FORM-STABILITY OF CILIATES IN RELATION TO PRESSURE AND TEMPERATURE[1]

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The first use of hydrostatic pressure as an experimental parameter in biological research is credited to Regnard (1884a, 1884b, 1884c) and Certes (1884), working independently. Both of these early workers were impressed by the variety of living forms that had been recovered by the deep-sea dredging expedition of the *Talisman* in 1882–1883. Regnard, particularly, became interested in the effects of pressure *per se*. He eliminated changes in the gaseous equilibria by applying the pressure directly to the aqueous medium and he studied the effects of pressures ranging up to 1000 atmospheres on a wide variety of small aquatic organisms. In the present connection, he observed that various ciliates, crowded in stagnant water and subjected to pressures of 600 to 1000 atmospheres for 10 minutes, became immobile and distended, and that ciliary movement stopped. Within two hours after decompression many of the organisms seemed to have recovered completely.

Ebbecke (1935, 1936) described the effect of pressure on paramecia. Exposure to pressures of 500 atmospheres for periods of from 10 to 30 minutes mainly effected a change in the shape of the ciliates, *i.e.*, their bodies became more spheroidal. At pressures of from 800 to 1000 atmospheres for periods extending from 5 to 30 minutes, the organisms became spheroidal and many underwent cytolysis. The rounding effects were reversible after a recovery period spanning several days. At 2000 atmospheres there was a drastic rounding of the cells, followed by complete cytolysis of all the organisms.

Hodapp and Luyet (1947) studied the mechanism of death of paramecia subjected to high hydrostatic pressure. They obtained a typical sigmoid curve of the percentage of paramecia killed by pressures varied systematically from 500–1200 atmospheres, each pressure being maintained for two minutes. At 950 atmospheres about 50 per cent of the cells were killed. Hodapp and Luyet also varied the time, the temperature, the rate at which the pressure was increased and decreased, and the age of the cultures, and found that the total compression time and the culture age were most important in relation to lethality. Temperature (between 10°–22° C.) and the rate of pressure increase were reported to have little or no effect upon lethality. In this connection, however, it should be realized that Hodapp and Luyet did not employ a windowed pressure chamber and could not observe the effects until after the organisms were removed from the chamber. Consequently, the compression and decompression effects could not be differentiated.

In 1934, D. E. S. Brown, studying frog muscle, first recognized the interrela-

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tionship between pressure and temperature in biological systems, and an appreciation of this important relationship did much to clarify subsequent investigations in the field. Further insight, particularly with reference to the problem of how pressure induces the solation of protoplasmic gels, was provided by a classification of gelational phenomena, published by Freundlich in 1937. Experiments by Heyman (1935, 1936) had shown that certain gel systems, represented by methyl cellulose, behave oppositely to gelatin. The methyl cellulose type of system displays a volume increment ($+\Delta V$) upon gelation; and Freundlich deduced that such gelations must be endergonic in nature. Then Marsland and Brown (1942) studied the sol-gel equilibria of myosin, methyl cellulose, and gelatin as affected by hydrostatic pressure and temperature. These experiments showed that myosin (*in vitro*), methyl cellulose, and protoplasmic gels, generally, must be placed in a common class of system because all undergo solation as a result of compression and cooling. Gelatin, on the other hand, gelates more firmly with cooling and compression and must represent a different class of system. These workers also emphasized the speed with which protoplasmic sol-gel equilibria may be shifted, particularly in fresh myosin preparations, and they postulated the intervention of an enzyme system (an ATP-ase complex) which likewise is sensitive to pressure and temperature.

The studies of Marsland (1950) demonstrated that the cortical plasmagel of dividing sea urchin eggs reacts to pressure-temperature treatments as do other intracellular gels. Landau *et al.* (1954), performing similar experiments on the plasmagel system of *Amoeba proteus*, came to the same conclusion, namely, that the gel system is weakened by higher pressures and lower temperatures, within the physiological range.

It is apparent, therefore, that pressure-temperature conditions may effect profound changes in cell structure and that such changes are determined, at least partly, by pressure-temperature effects upon the gelational state of the protoplasm. The purpose of the present work, accordingly, is to study the form-stability of two representative ciliates, Blepharisma and Paramecium, under systematically varied conditions of pressure and temperature. Generally, it has been supposed that the characteristic morphology and structural integrity of these organisms is maintained, not only by a tough, flexible surface pellicle, but also by a peripheral gelated layer of cytoplasm, often referred to as "ectoplasm." In the previous pressure studies on Paramecium, the organisms were not observed clearly during the compression period so that compression effects could not be distinguished from the effects of decompression; and in the previous studies on Blepharisma (Hirshfield *et al.*, 1957) no extensive variation in temperature was employed.

Materials and Methods

The ciliates, *Blepharisma undulans* and *Paramecium caudatum*, provided excellent material for this study because of their elongate shape which tends to become spheroidal when structural instability develops.

The original Blepharisma culture was obtained from Dr. H. I. Hirshfield; and the paramecia were derived from a mass culture maintained for many years at New York University. Both species were cultured in a lettuce-*Pseudomonas ovalis*

medium according to the method of Hirshfield (personal communication). The Blepharisma were maintained in an incubator at a temperature of 20° C., whereas the paramecia were kept at room temperature (25°–27° C.).

Periodically, single organisms were isolated and placed in the separate spots of a 12-spot Klein agglutination slide for five days. The contents of each depression were then transferred to a test-tube of lettuce medium which had been inoculated three days previously with *Pseudomonas ovalis.* Such Blepharisma clones were used for experimental purposes only from the seventh to the eleventh day after isolation. These gave fairly consistent data, but organisms from older cultures displayed a marked increase in pressure sensitivity. Paramecium cultures were more stable and gave fairly consistent results over a three-week period.

The constant-temperature housing, used with the pressure apparatus, has been described by Marsland (1950). The apparatus provides for a rapid build-up and release of pressure, and for constant microscopic observation while the organisms are under pressure. In the pressure bomb the protozoa were kept in view by confining them within a small plastic chamber (6.5 mm. diameter and 2 mm. depth) which was closed above and below by glass coverslips held in position by Lubriseal films.

Ten to thirty ciliates were placed in the chamber for each experiment, and the remaining volume (approximately 85 ml.) of the pressure chamber was filled with Brandwein solution. The duration of exposure to any given pressure was fifteen minutes. The pressures ranged from 7000 to 11,000 psi. and the temperatures employed were 12°, 15°, 20°, and 25° C. The organisms were counted several times during the progress of each experiment, and then at the end of the 15-minute period the percentage of cytolyzed individuals was determined. At least 60 Blepharisma in a total of four or more experiments were used at each pressure and temperature, at least in all critical ranges.

Results

Blepharisma: pressure-temperature effects on form-stability

Blepharisma from old mixed cultures were very sensitive to pressure. In such cultures (at 20° C.) 4000 psi. (lbs./in.^{2}) usually was sufficient to cause a rounding up of all the specimens and subsequently cytolysis occurred in over 75 per cent. Also, aging cloned cultures showed a steady decrease in resistance to pressure. In fact, during the fourth or fifth week after cloning, pressures of about 4000 psi. became sufficient to cause breakdown of the organisms, as was the case with the mixed cultures. Occasionally, young cloned cultures were found which displayed a similar super-sensitivity to the pressure-temperature conditions. Perhaps such clones were derived from a weak or aberrant individual. In any event they were not used for further experimentation.

Blepharisma from typical young cloned cultures showed little or no tendency to become rounded, regardless of the experimental temperature, until the pressure exceeded 7000 psi. Moreover, there was virtually no cytolysis within the 15-minute experimental period. At higher pressures, however, a number of the specimens first became rounded and then cytolyzed. Furthermore, the temperature of the

TABLE I

Percentage of cytolysis in Blepharisma after 15-minute exposure to various pressure-temperature conditions. These are the results of the individual experiments. In each experiment the number of lysed specimens is given in relation to the total number treated

$T°$ C.	psi.			
	8000	9000	10,000	11,000
12	10–20 13–25 8–15 31–60 = 52%	12–15 15–20 27–35 = 77%	17–18 = 100%	—
15	4–20 3–12 9–23 7–15 23–70 = 33%	6–10 7–13 10–20 10–15 8–13 11–17 52–88 = 59%	9–10 23–25 11–12 13–15 20–22 76–84 = 91%	15–15 = 100%
20	5–25 3–15 2–10 3–10 4–13 2–10 3–10 22–93 = 24%	7–15 5–15 6–18 8–20 5–15 4–11 4–11 39–105 = 37%	6–10 9–10 14–15 9–10 13–15 51–60 = 85%	14–15 8–8 15–15 37–38 = 100%
25	3–20 2–26 2–16 2–22 9–82 = 11%	3–10 4–11 4–14 4–10 3–10 6–15 24–70 = 34%	5–6 7–8 17–20 10–12 13–17 9–12 13–16 74–91 = 81%	15–15 = 100%

experiment had a distinct influence upon the percentage of suseptibility, as is shown in Table I.

The character of the rounding and of the subsequent cytolysis varied somewhat in relation to the intensity of the pressure treatment and to the experimental temperature. However, under *critical conditions*—which may be defined as any pressure-temperature combination which yields just 50 per cent cytolysis in 15 minutes—the reactions were generally similar. Thus it is possible to describe the variations which occurred under sub-critical, critical, and super-critical conditions which, respectively, yielded more and more cytolysis within the experimental time.

The rounding and cytolysis reactions under slightly sub-critical conditions (9000 psi./25° C.) are shown in Figure 1. Under such conditions, generally speaking, the shortening seldom exceeded 25 per cent of the original length; the number of rounded specimens increased only gradually during the experimental period; and the tapered anterior end of the organism tended to retain a fairly close semblance of its original architecture. Generally, motility was absent or at least drastically retarded in the rounded specimens.

Lysis, as was the case under all conditions studied, occurred only subsequent to the rounding reaction. Under sub-critical conditions the time of the lysis was distributed quite evenly throughout the test period. In each specimen, however, the lysis was sudden, sometimes being initiated in the tapered anterior end (Fig. 1, C), and sometimes in the swollen posterior half, near the contractile vacuole. It appeared to involve a sudden rupturing of the cell surface and a disruption of the cytoplasm into a number of rounded free-floating pieces (Fig. 1, D). Occasionally, some of these protoplasmic fragments become motile after pressure was released.

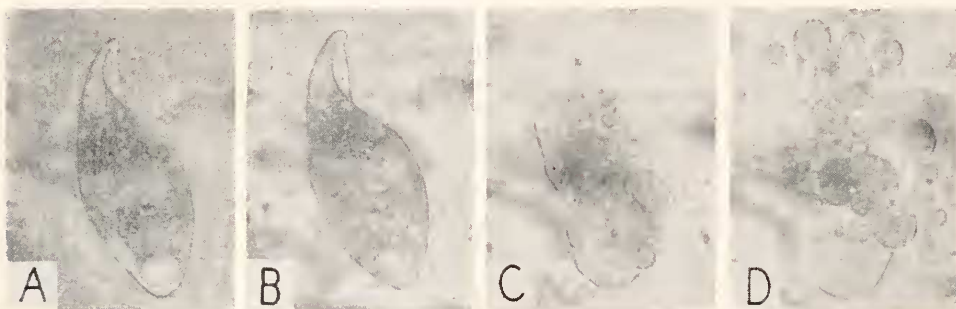

FIGURE 1. Blepharisma: rounding and cytolysis reactions under sub-critical conditions (9000 psi./25° C.). A and B: Gradual shortening of specimen; successive exposures taken 4 and 7 minutes after pressure build-up. C: Sudden cytolysis, exposure 30 seconds after B. D: Rounding up of cytoplasmic remnants, one minute after cytolysis. After decompression some of these fragments may become motile. Photographs retouched.

Under distinctly super-critical conditions, rounding and lysis developed rapidly and most of the susceptible specimens had reacted within the first five minutes. The shortening of specimens was distinctly greater, although often there was some persistence of the tapered anterior end up to the moment of lysis. The lysis was more complete; the protoplasmic fragments were smaller; these fragments showed less tendency to round up, and they did not develop motility subsequent to decompression.

Under intermediate conditions, in and around the critical range, the rounding and cytolysis reactions were intermediate in character.

Blepharisma: decompression effects

The sudden release of pressure, under critical or nearly critical conditions, gave rise, within two minutes, to an abrupt, further shortening of all the non-cytolyzed specimens, accompanied by a momentary stoppage of any persisting ciliary activity.

This shortening (Fig. 2) was more abrupt than the pressure-induced rounding. Immediately after shortening, a few specimens displayed sudden lysis, but this *decompression lysis* did not involve more than five per cent of the animals. In fact, most of the specimens regained their motility within some ten minutes, and after 30 to 200 minutes they presented a fairly normal form and appearance.

Blepharisma: pressure-temperature parameters of cytolysis

As may be seen in Table I, the percentage of cytolysis obtained at any given pressure represents a temperature-dependent value. The sensitivity to pressure cytolysis increases very definitely with decreasing temperature within the experimental range (25°–12° C.). This is shown more clearly when the data are plotted, as in Figure 3. Conversely, the resistance to pressure cytolysis increases with increasing temperature, as is shown in Figure 4. There it may be seen that the

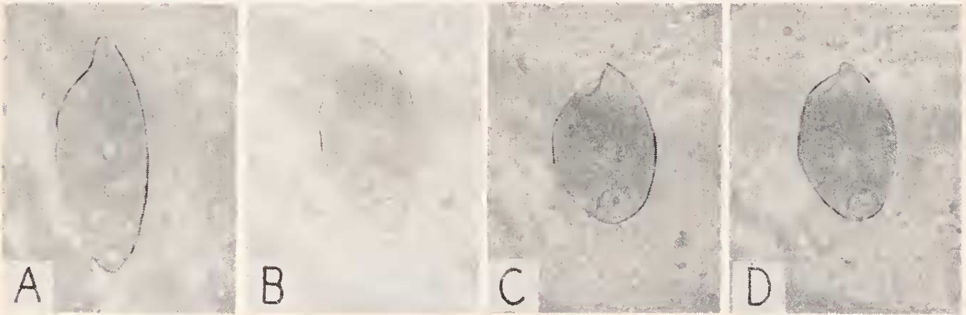

FIGURE 2. Blepharisma: shortening under pressure (slightly sub-critical conditions, *i.e.*, 9000 psi./25° C.) followed by rapid shortening after decompression. A: Exposure 13 minutes after pressure build-up. B: One minute after decompression. C: Two minutes later. D: Another two minutes later. Photographs retouched.

pressure which is just adequate to induce cytolysis in 50 per cent of the treated specimens increases regularly as the temperature increases, within the given range.

Blepharisma: pressure-centrifuge experiments

Quantitative measurements of the solational effects of pressure are difficult to obtain with Blepharisma. The pressure-centrifuge method, which has been used widely for other cells (Marsland, 1956), is not very suitable. The orientation of the specimens in the centrifugal field shows considerable variation, and the instability of form and cellular integrity at higher pressures gives further difficulty.

It was possible, however, to obtain qualitative data which showed unequivocally that pressure does induce solational changes in the cytoplasm of Blepharisma. Many of the specimens centrifuged for one minute at 5000 × gravity at 3000 psi. showed a distinct clearing of the centripetal half of the cell—by virtue of the centrifugal displacement of food vacuoles and other granular bodies—to a degree that was never found in control specimens, centrifuged simultaneously at atmospheric pressure.

Paramecium: comparative observations

Generally speaking, the pressure-temperature effects on Paramecium and Blepharisma were similar. However, there were two important differences: 1) Paramecium was distinctly more sensitive to pressure lysis, and 2) *decompression lysis,* which was almost negligible in Blepharisma, became very significant in Paramecium.

For Paramecium, the critical pressure for 50 per cent lysis was 2000–3000 psi. lower than for Blepharisma, at each of the two temperatures (20° and 25° C.) which were studied. Under such critical conditions (*e.g.,* 7000 psi./20° C.) the animals shortened moderately and displayed gradually diminishing, distinctly irregular locomotion, which ceased only if and when cytolysis occurred. Most of the cytolysis occurred during the last 5 minutes of the 15-minute compression period. Moreover, two somewhat different types of lysis were observed with roughly equal frequency. One type seemed to involve a detachment of the pellicle, with the formation of one or more large hyaline blisters which later broke, liberating the deeper granular cytoplasm (Fig. 5). The other type, in contrast, seemed to

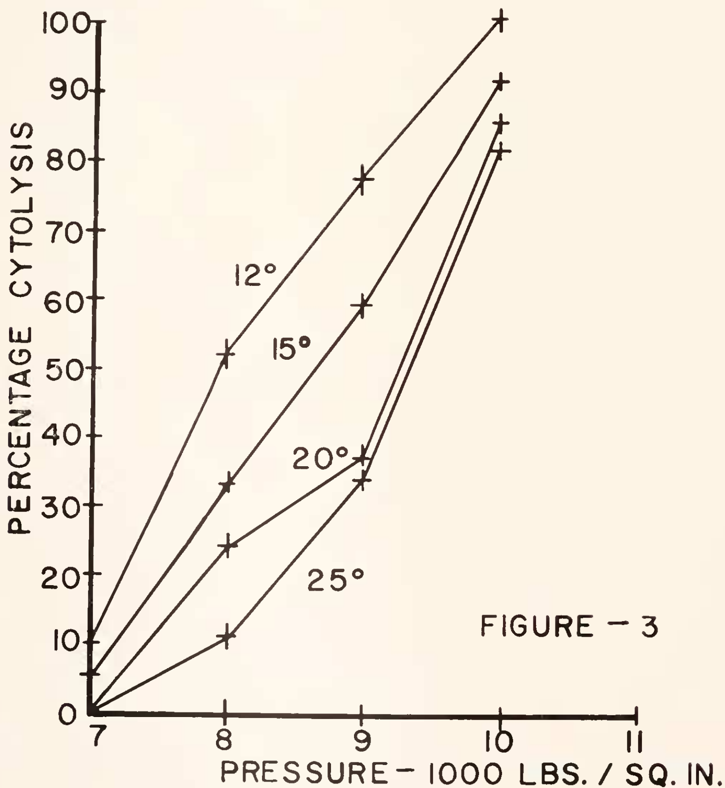

FIGURE 3. Blepharisma: percentage of cytolysis as a function of pressure, at four temperatures. Cytolyzed cells were counted exactly 15 minutes subsequent to pressure build-up.

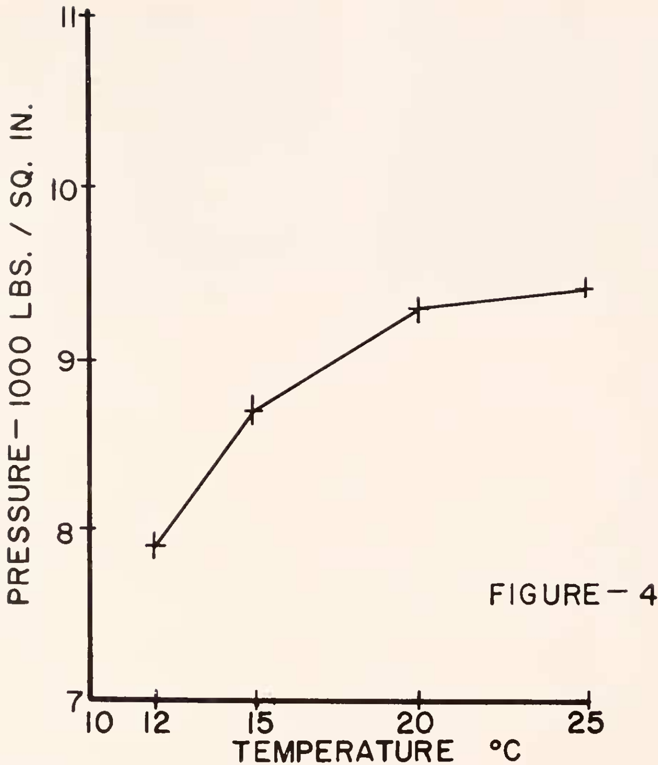

FIGURE 4. Blepharisma: critical pressure yielding 50 per cent cytolysis after 15-minute exposure, plotted as a function of temperature. The data of this figure are derived from Figure 3.

represent a more generalized breakdown of the cell surface in either the anterior or posterior half of the animal, with a less abrupt scattering of the granular cytoplasm (Fig. 6). With Paramecium, moreover, regardless of the conditions or type of cytolysis, there was very little tendency for the cytoplasmic remnants to round up, or to wall themselves off from the surrounding medium.

The decompression lysis under critical conditions usually involved more than half of the surviving specimens, particularly when the decompression was rapid (within one second). Sudden decompression was followed within about two minutes by an abrupt further shortening of all surviving specimens, followed immediately by a generalized cytolysis of the majority. Specimens that escaped cytolysis, on the other hand, gradually regained normal form and motility within 2–3 hours.

Under super-critical compression (8000 psi./20° C.), the degree of rounding was greater; and most of the lysis, which involved more than 60 per cent of the specimens, occurred during the first ten minutes of the compression period. Then, following rapid decompression, *all* surviving specimens shortened still more and quickly underwent cytolysis.

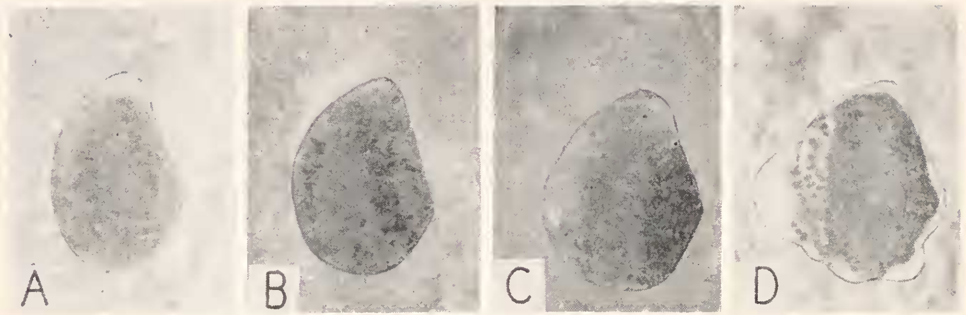

FIGURE 5. Paramecium: one type of pressure cytolysis (8000 psi./25° C.). A and B: Shortened specimen, photographed 9 and 10 minutes after compression. C: Two minutes later, cytolysis starting. D: 30 seconds later, showing hyaline blisters which are about to break. Photographs retouched.

Distinctly sub-critical conditions (5000 psi./20° C.) produced no clearly defined effects; but at 6000 psi. a slight degree of shortening was noted, although locomotion appeared to continue in normal manner. Abrupt decompression, under these conditions, produced a further sudden shortening of the specimens, but there was no cytolysis and usually the animals regained their normal form within one hour.

DISCUSSION

The problem of how pressure exerts its effects upon cellular systems has been approached from several angles. Regnard (1891) interpreted his results in terms of an imbibition of water by the ciliated cells. However, since no volume increase can be found in pressurized cells, this hypothesis has not been pursued. Hodapp and Luyet (1947) suggested a disturbance of the permeability mechanism and an injury of the neuromotor apparatus as the main factors involved in pressure lysis. However, as stated previously, they were unable to observe the organisms during the pressure period. As to their findings that temperature and the rate of application and release of pressure had no effect on lethality, at the high pressures they employed, it is probable that the decompression effects were very drastic and negated these variables.

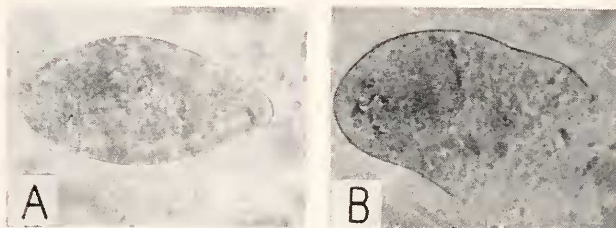

FIGURE 6. Paramecium: another more generalized type of pressure lysis (9000 psi./25° C.). A: Shortened intact specimen 5 minutes after pressure build-up. B: 5 minutes later, sudden cytolysis initially involving all of the anterior half of the specimen. Photographs retouched.

Pressure-temperature effects on cell form

A more fruitful approach, perhaps, is to interpret the observed effects on the cell form and integrity of Blepharisma and Paramecium in terms of the now well established action of pressure and temperature upon intracellular gel structures. To some extent this approach has been adopted by Ebbecke (1936), who had, however, very little experimental evidence. Moreover, Ebbecke postulated that pressure exerts its effect upon the gel system indirectly via an action upon cell metabolism, rather than directly *and* indirectly, as proposed by Marsland and Brown (1942).

It now seems reasonable to assume that the cortical cytoplasm of ciliates, immediately subjacent to the pellicle, is firmly gelated and that this plasmagel layer plays a significant role in helping to maintain the unique form of the particular species. Also, it seems possible that the plasmagel layer of the ciliate may possess contractile properties which can be instrumental in producing changes of form and orientation during normal locomotion.

Experimental evidence in regard to the foregoing question is not very extensive, however. A plasmagel structure is indicated, to be sure, by the fact that the peripheral layer of cytoplasm, in which the trichocysts are lodged, does not become involved in the protoplasmic streaming when cyclosis occurs. Also, it is frequently observed, not only in the pressure-temperature experiments, but also when Paramecium and other ciliates are exposed to toxic substances or merely flattened under a coverslip, that the pellicle may peel away from the subjacent cytoplasm and form a hyaline blister of large or smaller size. When this happens the granular cytoplasm, from which the pellicle has become detached, may persist, retaining its stability for a minute or two. Then it disintegrates and pours forth its granular components into the hyaline fluid which fills the blister.

All the evidence of the present experiments indicates that the shortening and rounding of the cells induced by suitably high pressure and modified by temperature are mediated by a solation of the plasmagel layer. Qualitatively, the susceptibility of this gel to pressure solation is established by the pressure-centrifuge experiments and quantitatively the pressure-temperature parameters of this gel system are very similar to those which have been established in various other protoplasmic gels (Marsland, 1956). Apparently a shortening and rounding of these elongate ciliated cells occur, under the agency of tensional forces in the cell surface, whenever the subjacent plasmagel structure is weakened below a certain critical resistance level.

Pressure-temperature lysis

Pressure lysis, apparently, is always preceded by a rounding of the cells; and, generally speaking, the more drastic the rounding the greater is the lysis tendency. It seems likely, therefore, that cell form and cell integrity may be determined by similar underlying factors.

A firmly maintained plasmagel structure would serve, most probably, not only to stabilize the total form of the cell, but also to preserve the orientation and spatial configuration of many of the microscopic and submicroscopic constituents of cell structure. Moreover, if the solation is drastic enough to allow for a rounding of the cell, the rounding itself tends to disturb and disorient the configuration of the

protoplasmic constituents. Cytolysis, perhaps, may involve a detachment of the pellicle from the subjacent plasmagel, with a concomitant disarrangement of the ciliary origins and trichocysts, or it may involve some other type of disorientation. In any event, it seems to occur whenever drastic solation occurs. Thus it is not surprising to note that preliminary dosages of UV-irradiation, utilizing wave-lengths which have a primary effect upon the proteins of the peripheral cytoplasm, predispose Blepharisma to pressure cytolysis, presumably as a result of a weakening effect upon the plasmagel structure (Hirshfield *et al.,* 1957).

Decompression lysis

This phenomenon, which was particularly conspicuous in Paramecium, can be interpreted, perhaps, in terms of a rapid post-pressure reconstruction and contraction of the plasmagel system. A similar phenomenon, in fact, has been described for Amoeba by Landau, Zimmerman and Marsland (1954). Many studies have shown that pressure solation is rapidly reversible upon decompression and the Amoeba study indicates that the newly reconstituted gel system tends to contract sharply, presumably as a result of an accumulation of metabolites which are not fully utilized during the pressure period (Landau *et al.,* 1954). In any event, both Blepharisma and Paramecium always showed an abrupt contraction about two minutes after release from any extensive critical or super-critical pressure-temperature treatment and, particularly in the case of Paramecium, this abrupt contraction was very frequently accompanied by cytolysis. Precisely why cytolysis should occur under these circumstances is problematical. It may be supposed, however, that such a contraction would tend to disrupt the surface architecture of the cell, especially if it occurs before a proper stabilization of the cell structure has occurred. Furthermore, these observations indicate that the peripheral gelated cytoplasm of the ciliate displays a potential contractility, and that this layer may play a role in effecting changes of form and orientation, during locomotion, and in performing the work of cell division.

Metabolic relationships

The increasing susceptibility of older cloned cultures in regard to pressure-temperature cytolysis raises some interesting questions. A continued source of metabolic energy appears to be necessary for the maintenance of protoplasmic gel structures (see Marsland, 1956) ; but why should such structures tend to be weaker in aging clones? Lettré (1952) has suggested that cell form and stability may be dependent upon the level of ATP reserve in the cell, but why should this tend to diminish with age? ATP-sensitive proteins, capable of forming potentially contractile gel systems, seem to be present in various relatively unspecialized cells—in the slime mold (Loewy, 1952 and Ts'o *et al.,* 1956), in sea urchin eggs (Mirsky, 1936), in fibroblasts and other tissue cells (Weber, 1955 and Hoffman-Berling, 1954), and in Amoeba (Landau *et al.,* 1954). At present, however, it is entirely problematical as to whether age-changes in the gel structure result from changes in metabolism, changes in the constituent proteins or, at least partly, from other unknown changes.

Summary

1. Two ciliates, *Blepharisma undulans* and *Paramecium caudatum,* were studied with reference to form stability and integrity (resistance to cytolysis) under varying conditions of hydrostatic pressure (up to 10,000 lbs./in.^{2}) and of temperature (12°–25° C.).

2. At lower pressures the specimens retained their elongate form, but at higher levels, depending on temperature, species, and age of the cloned cultures, the cells gradually become shorter and more rounded. Following this form change, cytolysis occurred in a varying percentage of the specimens. Older cloned cultures showed a greater and more variable susceptibility to the pressure-temperature effects, so that selected younger cultures were used for the quantitative evaluations.

3. For Blepharisma, the critical pressure, which gave 50 per cent cytolysis within a 15-minute compression period, displayed a distinct temperature dependence, being 8000 psi. at 12° C., 8700 at 15° C., 9200 at 20° C., and 9300 at 25° C. Paramecium, in contrast, showed a distinctly greater sensitivity, the critical pressure for 50 per cent cytolysis at 20° C. being some 2000 psi. lower than for Blepharisma.

4. Rapid decompression, following any critical or super-critical pressure treatment, produced an abrupt further shortening (contraction) of the specimens, accompanied by a cytolysis of some of the previously resistant individuals. For Blepharisma, decompression cytolysis involved only about 5 per cent of the animals. Paramecium, however, was much more sensitive and virtually 100 per cent became involved.

5. An interpretation of these changes in cell form and integrity is given in terms of pressure-temperature effects upon protoplasmic gel structure, particularly with reference to the solation of the peripheral plasmagel layer of the cytoplasm.

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